

Absorption of calcium from tortilla meals prepared from low-phytate maize¹⁻³

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ABSTRACT

Background: Calcium fortification of maize has been achieved for millennia in Central America by the process of nixtamalization. Bioavailability of calcium is, however, compromised by phytate, which is present in large quantities in maize kernels and is only modestly reduced by nixtamalization.

Objective: The objective was to compare the absorption of calcium from tortilla meals prepared from low-phytate maize with that from meals prepared from maize with typical phytate content.

Design: At 1-mo intervals, 5 healthy adult women were fed 2 test meals of ≈ 140 g tortillas in lieu of breakfast. On one occasion, the tortillas were prepared from maize with $\approx 60\%$ phytate reduction, and, on the other occasion, they were prepared from the matching isohybrid wild-type maize. Beginning midway through the test meal, ^{44}Ca (0.3 mg/kg body wt) was administered in water as an extrinsic label; ^{42}Ca (0.06 mg/kg body wt) was administered intravenously immediately after the test meal. Isotope ratios of ^{42}Ca to ^{43}Ca and of ^{44}Ca to ^{43}Ca were measured by inductively coupled plasma mass spectrometry in urine collected as an 8-h pool from the period 16–24 h after intravenous tracer administration and prepared by the oxalate precipitation method. Fractional absorption of calcium was determined by using a dual-isotope ratio technique.

Results: Mean fractional absorption of calcium from tortillas prepared from the low-phytate maize (0.50 ± 0.03) was significantly ($P = 0.003$) greater than that from tortillas prepared from the control maize (0.35 ± 0.07).

Conclusion: The increase in the quantity of calcium absorbed could be of practical importance for calcium nutriture when the intake of dairy products is limited. *Am J Clin Nutr* 2005;82:84–7.

KEY WORDS Maize, phytate, low-phytate maize alleles, tortillas, calcium absorption

INTRODUCTION

Cereal grains contain only negligible quantities of calcium. However, substantial quantities of this mineral are added to maize during the process of nixtamalization, or liming, which is the traditional method of processing maize in the preparation of tortillas in Central America (1). From the perspective of calcium nutriture, nixtamalization can be regarded as an early example of mineral fortification of a major food staple in Central America. Depending on the extent to which this added calcium is bioavailable, maize tortillas may provide a major source of calcium for persons whose intake of dairy products is limited.

Whereas the absorption of endogenous iron from a low-phytate acid maize was modestly higher than that from control maize (2), substitution of a low-phytate-acid maize has not been associated with any increase in the absorption of iron that was added to maize flour as a fortificant (3). These observations have given additional impetus to determining whether the substitution of low-phytate-acid maize affects the bioavailability of calcium in maize tortillas that is derived almost entirely from the process of nixtamalization.

The hypothesis tested in this study was that fractional calcium absorption (FCA) from tortilla meals prepared from maize with $\approx 60\%$ phytate reduction is significantly greater than from tortilla meals prepared from wild-type control maize.

SUBJECTS AND METHODS

Study design

Fractional absorption of an extrinsic calcium stable isotope label by healthy adults was measured when administered with a test meal of tortillas prepared from low-phytate maize. In a cross-over design with a washout period of >4 wk, fractional calcium absorption from a test meal of tortillas prepared from a low-phytate maize was compared with that from a tortilla test meal prepared from the isohybrid wild-type control maize.

Calcium absorption was measured using an extrinsic calcium stable isotope label by a dual-isotope tracer ratio technique based

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on measurements in the urine of the dose-adjusted ratio of enrichment with the oral extrinsic label to that for a second calcium stable isotope tracer administered intravenously (4).

Subjects

The subjects were 5 healthy free-living volunteer adult women aged 22–29 y who were recruited from the University of Colorado Health Sciences Center community by word of mouth. We included women of childbearing age who had normal BMI and an omnivore diet and who were willing to eat maize-only breakfasts on 2 consecutive days. Exclusion criteria included pregnancy, lactation, use of a multivitamin or mineral supplement, regular consumption of fortified breakfast cereals or energy bars, regular use of medicines that affect absorption, and the presence of any chronic or acute illness.

Written informed consent was provided by all subjects. The protocol was approved by the Colorado Multiple Institutional Review Board of the University of Colorado Health Sciences Center.

Source of maize and preparation and administration of test meals

Test meals were prepared from low-phytate-acid maize (*lpa1-1*) that has $\approx 60\%$ phytate reduction. This maize was provided by Pioneer Hi-Bred Inc (Dupont, Johnston, IA), which cultivated it under a cooperative research and development agreement with the US Department of Agriculture. The isohybrid wild-type maize with normal phytate content was also provided by Pioneer Hi-Bred Inc and was grown in the same location.

For the preparation of tortillas, 10 L water was added to 450 g maize kernels, and the mixture was brought to the boil. Powdered limestone (5 g, or 1 tsp) was added, and the mixture was stirred. The maize mixture was left to simmer for 4 h, after which it was drained and spread out on a towel to dry for 3 h. The nixtamalized maize was then ground in a food processor, rolled into ≈ 4 -cm diameter balls, dipped in corn oil, and flattened. The tortillas were cooked on a greased skillet for 1 min. Each meal consisted of 5 of these tortillas, which weighed ≈ 35 g.

After an overnight fast, test meals were administered at ≈ 0800 in the presence of one of the investigators. Three subjects received tortillas prepared from the *lpa1-1*, and the other 2 received tortillas prepared from the isohybrid wild-type maize. After a washout period of 4 wk, subjects consumed the alternative test meal.

Isotope preparation

Enriched ^{42}Ca and ^{44}Ca stable isotopes were obtained from Trace Science International Inc (Richmond Hill, Canada) as carbonate. Enriched ^{44}Ca was used as the orally administered tracer, and ^{42}Ca was used as the intravenously administered tracer. Calcium carbonate was dissolved by adding drops of concentrated hydrochloric acid. The oral solution was prepared at a calcium concentration of 0.063 mol/L by dilution with Milli-Q water (Millipore Systems, Bedford, MA), and the intravenous solution was prepared at a calcium concentration of 0.01 mol/L 0.45% sterile sodium chloride. We adjusted the oral solution to pH 5.0 and the intravenous solution to pH 6.0 with sodium hydroxide. The solutions were filtered through a 0.2- μm filter to remove pyrogens. Sterile techniques were used to prepare doses

for intravenous administration. Calcium concentrations were determined by atomic absorption spectrophotometry with application of correction factors for atomic weight. The oral solution was stored in plastic tubes, and the intravenous solution was stored in sealed sterile vials. The intravenous dose was tested for pyrogens immediately before use.

Isotope administration

An accurately weighed quantity of ^{44}Ca (≈ 0.3 mg Ca/kg body wt) was administered orally in water starting approximately halfway through the test meal. We have used this method extensively to administer stable isotope tracers of zinc (5). An accurately weighed quantity of ^{42}Ca (≈ 0.06 mg Ca/kg body wt) was administered intravenously over a 10-min interval immediately after the test meal. Administration was performed over a 5-min interval with the use of a 10-mL syringe, a 3-way stopcock, and a scalp vein needle placed in a superficial forearm vein. The syringe was flushed twice with sterile normal saline.

Sample collection, preparation, and analysis

Participants were instructed to completely empty the bladder immediately before administration of the intravenous isotope tracer. All urine was collected for an 8-h period beginning 16 h after the administration of the intravenous tracer. Urine was collected directly into an acid-washed plastic bottle. Volumes were measured, and a 50-mL aliquot was then stored at -20°C .

For analysis, 5 mL urine from the period 16–24 h after isotope administration was purified by the oxalate precipitation method (6). Urine was first centrifuged to remove particulates, saturated ammonium oxalate was adjusted to pH 8.0 with NH_4OH , and 1.2 mL saturated ammonium oxalate was added to the urine. After thorough mixing, the sample was left at room temperature overnight. It was then centrifuged at $1700 \times g$ for 15 min at room temperature, and the supernatant fluid was decanted. The precipitate was washed twice with Milli-Q water and dissolved in 4 mL of 2% HNO_3 . We prepared 8 mL of 1 ppm calcium solution in 2% HNO_3 from each sample. Isotope ratios of ^{42}Ca to ^{43}Ca and ^{44}Ca to ^{43}Ca were measured with the use of an inductively coupled plasma mass spectrometer (ICP-MS) (Plasma Quad 3; VG Elemental, Winsford, United Kingdom). Each sample was introduced into the ICP-MS by using an autosampler (ASX-500 Model 510; CETAC, Omaha, NE) and peristaltic pump (Perimax 12; CPETEC, Erding, Germany). To minimize argon-derived isobaric and polyatomic interference, the instrument was operated in the cool plasma mode. Instrumental settings for cool-plasma-mode operation are given in **Table 1**.

Two-percent HNO_3 and a 1 ppm natural abundance calcium standard were used to optimize ICP-MS tuning to attain the lowest ion count for the 2% HNO_3 blank and the highest count rate for the natural abundance calcium standard. With a ^{44}Ca count rate of 300 000–400 000 per second from the 1 ppm natural abundance calcium standard, the ICP-MS can be tuned to produce a 2% HNO_3 signal of $<0.4\%$ of the 1 ppm calcium signal for isotopes ^{42}Ca , ^{43}Ca , and ^{44}Ca . A natural abundance calcium standard was analyzed after every 6 urine samples, and 2% HNO_3 was analyzed after every 12 samples. The results were used to reduce any effect of instrumental drift on measured ratios. The 2% HNO_3 count rate was subtracted from urine sample count rates. The relative SD for the analysis of 10 replicates was $<0.3\%$ for both ^{42}Ca : ^{43}Ca and ^{44}Ca : ^{43}Ca . The calcium tracer enrichments were calculated from the measured isotope ratios by using



TABLE 1

Inductively coupled plasma mass spectrometer and calcium isotope data-acquisition variables

Variable	Setting
Argon gas flow (L/min)	
Outer	12.0
Intermediate	0.50
Nebulizer	1.00
Forward power (W)	700
Temperature of pneumatic nebulizer (°C)	4
Sample flow rate (mL/min)	1
Data acquisition	
Dwell time (ms)	
⁴² Ca	4
⁴³ Ca	5
⁴⁴ Ca	3
Points per peak (n)	1
Sweeps (n)	2280
Dead time (ns)	50
Replicates (n)	10

an algorithm that takes into account the contribution to each isotope signal of calcium from both isotopically enriched tracers and the naturally occurring calcium in the sample. For each isotope tracer used, enrichment is defined as all the calcium in the sample from that particular tracer divided by the total amount of calcium in the sample.

The weights of the test meals consumed were recorded. Tortillas from each test meal were collected and homogenized. Weighed aliquots were digested by heating samples to 450 °C for 24 h, wet ashing on a hot plate with concentrated HNO₃, and then ashing at 450 °C for an additional 24 h, and the calcium content was determined by atomic absorption spectrophotometry (7). HPLC was used for direct measurement of phytate (8).

Data processing and statistical analysis

Data were analyzed by using GRAPHPAD PRISM for WINDOWS software (version 4.00; GraphPad Software, San Diego, CA; www.graphpad.com). Mean (\pm SD) calcium intake was calculated per gram of tortilla and per test meal. The molar calcium:phytate of the test and control meals was calculated.

Mean FCA was determined for the low-phytate and the wild-type maize tortilla meals. Calcium absorption from the low-phytate maize tortilla meals was compared with that from the wild-type control maize tortilla meals by using a two-tailed, paired comparison *t* test.

RESULTS

Results are presented as means \pm SDs. The calcium contents of the low-phytate and control tortillas did not differ significantly ($P = 0.90$), and the results were combined to give a mean calcium concentration of 1.00 ± 0.39 mg/g wet weight tortilla. The quantity of tortilla consumed averaged 140 g/test meal, and the mean calcium intake was 140 mg. Phytate concentrations in the low-phytate and control tortillas were 1.56 and 3.0 mg/g, respectively, which gave corresponding molar calcium:phytate of 10.9 and 4.9.

All individual subjects had significantly higher FCA from the *lpa1-1* maize tortilla meals than from the control maize tortilla meals (Figure 1). Mean FCA from the low-phytate maize tortilla meals was 0.50 ± 0.03 compared with a mean of 0.35 ± 0.07

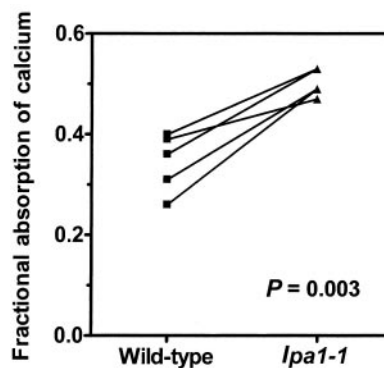


FIGURE 1. Comparison of fractional calcium absorption from tortillas prepared with a 60% phytate-reduced maize (low-phytate-acid; *lpa1-1*) or isohybrid wild-type maize in individual subjects.


from the tortilla meals prepared from the isohybrid wild-type control maize ($P = 0.003$).

DISCUSSION

Phytate strongly chelates calcium. Calcium-phytate complexes are quite soluble at an acidic pH but have only very limited solubility at the neutral or alkaline pH of the small intestine (9). Phytate was reported as early as 1934 to have an inhibitory effect on calcium absorption in rats (10). Subsequent reports of the effects of phytate on calcium absorption in rodents were conflicting (9–17), which, it was suggested, may be due to the presence of intestinal phytases in the rodent (18). The substitution of low-phytate cereal grains for grains with a typical phytate content in feed for chicks, pigs, and fish has been associated with improved calcium nutrition in each species (19–21). The destruction of phytate in wheat flour was shown in 1942 to improve calcium retention in humans (22). The inhibitory effect of phytate on calcium absorption in humans has subsequently been confirmed (23, 24), and calcium absorption from low-phytate soybeans was reported to be significantly higher than that from high-phytate soybeans (18). Even in humans, however, the adverse effect of phytate on calcium absorption has excited no (25) or relatively little (26) attention in reviews.

In the current study, the difference between the absorption of calcium from tortilla meals prepared with the low-phytate maize and that from meals prepared with maize with a typical phytate content was similar but of greater magnitude than the difference between the absorption of calcium from low-phytate and high-phytate whole cooked soybeans (18). The calcium content of the tortillas prepared for this study was comparable to that found by other investigators (1). With a typical tortilla wet weight of ≈ 40 g, the quantity of calcium ingested with each tortilla was ≈ 40 mg. In our own experience in the western highlands of Guatemala, adult women typically consume ≥ 15 –20 tortillas/d (weight: ≈ 40 g each), which provide ≈ 500 mg calcium/d. The increased absorption found in the low-phytate maize tortillas would contribute an additional 6 mg Ca absorbed per tortilla or 90–120 mg Ca/d for typical maize intakes. Thus, when there is a limited intake of dairy products, tortillas provide the major source of dietary calcium. This calcium alone, however, is not typically sufficient to match the adequate intake recommended by the Institute of Medicine's Food and Nutrition Board (27), which states that any strategy that increases the bioavailability of this

calcium will make a useful contribution to calcium status. Even in North America, tortillas prepared from nixtamalized maize can provide a useful alternative source of calcium for subjects who do not consume dairy products, and the superior absorption of calcium from a low-phytate maize could be advantageous.

We showed previously that fractional absorption of zinc from tortillas prepared from maize with 2 different amounts of phytate reduction (but probably a mutation of the same allele) is higher than that from tortillas prepared from the corresponding wild-type maize with typical phytate content (28, 29). Others have found a modest increase in iron absorption (2). Especially when taken together, these data encourage the evaluation of the efficacy and effectiveness of a change in agricultural practice—ie, the use of low-phytate maize—to improve mineral nutriture in low-income populations that depend on maize as their principal food staple. 

KMH, NFK, JLW, SL, LVM, and VR participated in study design and data interpretation. SL and KLP were responsible for laboratory analyses. Data were analyzed by JLW. The manuscript was drafted by KM. None of the authors had any personal or financial conflicts of interest.

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